REMARKS

Early and favorable action on the merits is courteously awaited. The following pages show the VERSION WITH MARKINGS TO SHOW CHANGES MADE.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

A METHOD FOR MODULATING METABOLISM OF [NITRIC] <u>NITROGEN</u> OXIDES, A COMPOSITION FOR REALIZING THEREOF (VARIANTS) AND A METHOD FOR EFFECTING ORGANISM OF A PATIENT IN NEED OF CORRECTING METABOLISM OF [NITRIC] <u>NITROGEN</u> OXIDES.

IN THE SPECIFICATION:

On page 1, cancel the second paragraph and insert therefore

--Nitric oxide (NO) is the most important inorganic metabolite of all higher animals [1-5]. It takes part in controlling diameter of blood vessels [3] and septic shock [6], in transmission and storage of information [4]; it is an initial product for the synthesis of peroxynitrite that kills pathogenic bacteria and cancer cells [7, 8] and acts as an absorber of free radicals [9, 10]. In mammals NO formation results from arginine oxidation by oxygen as affected by NO-synthases [4, 11] and NO degradation occurs in further oxidation. Metabolism disorders of [nitric] <u>nitrogen</u> oxides causes a number of diseases; on the contrary, the methods that allow correcting metabolism of [nitric] <u>nitrogen</u> oxides (for example a physiological NO donor, nitroglycerin that is used in heart failure) are effective for preventing and treating numerous pathological conditions. In a gas phase NO is oxidized by oxygen to NO_2. In diluted aqueous solutions two main competing NO oxidation routes simulating in vivo conditions are known: (1) trielectronic oxidation when complexes of transitional metals,--.

On page 2, cancel the second full paragraph and insert therefore

--NO donors, such as organic nitrates, inhibitors of NO-synthases, such as nitroarginine and enzymes destructing arginine (the NO precursor) such as arginases and others, are used for correcting metabolism of [nitric] nitrogen oxides [5].--

On page 4, cancel the second and fourth full paragraphs and insert therefore

--The prior art does not teach the way of modulating metabolism of [nitric] nitrogen oxides is known from (i.e. accelerating or slowing down NO oxidation reaction and subsequent conversions) in the initially heterogeneous system (in one of the practically important cases in blood) without changing neither the amounts of reacting substances nor temperature and pressure in the system. The methods that allow changing relation between the reaction products of mono- and tri-electronic oxidation without adding additional reagents are also unknown.

The peptide Met-Glu-His-Phe-Pro-Gly-Pro being the base of pharmaceutical preparations of nootropic action is known [23]. The effect thereof on [renitrosation] transnitrosation is unknown.--.

On page 5 cancel the first and second full paragraphs and insert therefore

--While the therapeutic effects of the Russian steam bath and sauna are known but the character and mechanisms of their effect on metabolism of [nitric] nitrogen oxides are still unknown.

Disclosure of the invention

The suggested technical solution on modulating metabolism of [nitric] <u>nitrogen</u> oxides by varying NO oxidation rate consists in changing the reaction medium so that at least one novel phase would appear therein and/or the ratio between the volumes of the phases would change and/or the values of [distribution] <u>partition</u> coefficients Q_{NO} and/or Q_{O2} at least for one pair of phases would change so that the value of the

expression

[

$$H = \frac{\sum_{i=1}^{i=n-1} k_i Q_{NO,i}^{2} Q_{O_2,i} x_i + k_n \left(1 - \sum_{i=1}^{i=n-1} x_i\right)}{\left(1 + \sum_{i=1}^{i=n-1} Q_{NO,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)^2 \left(1 + \sum_{i=1}^{i=n-1} Q_{O_2,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)}$$

]

$$H = \frac{1+\sum_{i=1}^{i=n-1}\frac{k_i}{k_n}Q_{NO,i}^2 Q_{O_2,i}x_i - \sum_{i=1}^{i=n-1}x_i}{\left(1+\sum_{i=1}^{i=n-1}Q_{NO,i}x_i - \sum_{i=1}^{i=n-1}x_i\right)^2\left(1+\sum_{i=1}^{i=n-1}Q_{O_2,i}x_i - \sum_{i=1}^{i=n-1}x_i\right)}$$

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where H is acceleration of NO oxidation reaction with oxygen in a heterogeneous n-phasic system as compared to the least hydrophobic (aqueous) phase, k_i is the reaction rate constant in i phase, Q_{Noi}, Q_{O2i}- is a equilibrated [distribution] partition coefficient of NO and O_2 in i phase, x_i is a portion of i phase in a total volume,

describing a total reaction (2) acceleration trough the whole system (in all phases) would change.--.

On page 7, cancel the second full paragraph and insert therefore

--A simultaneous increase in the rate of radical reactions and trielectronic NO oxidation with formation of nitrate is the above disclosed general method's peculiarity which in some cases proves to be advantageous and in a number of cases it is a drawback. The reason for this event consists in the fact that N_2O_3 salvation that promotes stabilization thereof in hydrophobic phases is lower than in water, in particular in the phases with a high level of fluororganic compounds. Accordingly the system equilibration of higher [nitric] nitrogen oxides shifts towards NO_2 and N_2O_4 formation. The former promotes the course of radical reactions including nitrotyrosine

formation, the latter enters reactions of electrophylic nitrosation nitrate being the second product thereof (see Fig.2).--.

Cancel page 8 and insert therefore

--necessity, several modifications are suggested in the claimed group of inventions. Possibility of controlling the time of affecting metabolism of [nitric] <u>nitrogen</u> oxides is provided by using perfluorocompounds of different molecular weight and structure, said compounds controlling elimination rate thereof from the body.

According to the suggested method, in order to shift equilibration towards N_2O_3 [renitrosation] <u>transnitrosation</u> catalysts that promote the course of N_2O_3 reactions and other nitrosating agents (such as N-nitrosotriptophane comprised in proteins with nitrosation targets in particular water (4), thiols (5) or amines) are additionally introduced into the heterogeneous medium in which NO oxidation occurs. The necessity of simultaneous administering several catalysts and/or inhibitors can be caused by the presence of many parallel reactions since catalytic effects for each of the catalyst/reaction pairs are individual. NO oxidation rate according to the reaction (2) remains unchanged but the steady state N_2O_3 concentration dropping as the degradation rate thereof increases. Accordingly steady state concentrations of NO_2 and N_2O_4 being in a dynamic equilibration therewith and the rates of radical reactions and nitrate formation drop. These catalysts and inhibitors can be administered into both aqueous phase and micellars of hydrophobic phase. Intranasal administering peptide catalysts of [renitrosation]

transnitrosation which rapidly getting into cerebral tissues will promote protection from damages of the specific binding sites for example can accompany Bath procedure.--.

On page 9, cancel the second paragraph and insert therefore

--The third way consists in adding into the reaction mixture the targets capable of penetrating into hydrophobic phases, to nitrosate therein and to eliminate nitrous group into aqueous phase. Due to the possibility of [renitrosation] transnitrosation not only for N_2O_3 but also for other nitrosocompounds in particular nitrosothiols and nitrosoamines, thiols and amines can perform such transport function. However, since nitrosoamines can be cancerogenic, thiols are preferable.--.

On page 10, cancel the first full paragraph and insert therefore

--The sixth way of equilibration shifting toward monoelectronic oxidation is increase in efficient nitrosation targets in the aqueous phase, the local N_2O_3 concentration at the interface of phases falling due to chemical reactions that results in formation of a steeper gradient and accelerating diffusion and transport of higher [nitric] nitrogen oxides from the hydrophobic phase into the aqueous one so that the steady state NO_2 and N_2O_4 concentration decrease. In one option of this method nitrosation on amine group of the targets if followed by split off a water molecule and degrade with release of free nitrogen. Thus NO-equivalent (an NO oxidation product) is irreversibly removed from the system (unlike nitrite and nitrothiols

that are capable of repeated reduction into NO - see Fig.2) that results in decrease in a total pool of NO-equivalents in the system. Biologically compatible non-toxic amides with unsubstituted amine group, urea, salts of carbamic, sulfaminic and amidophosphoric acids and hydrophobic primary amines can be used as the targets of such type for reactions in anhydrous phases.--.

Cancel page 11 and insert therefore

--Shifting equilibration of [nitric] nitrogen oxides towards NO_2 and N_2O_4 (i.e. increase in the proportion of radical reactions and trielectronic oxidation) is achieved by rise in concentration of oxygen and [renitrosation] transnitrosation inhibitors, by elevation of temperature, by decrease in area of interface surface between phases, by lowering concentration of the efficient targets and by excluding hydrophobic targets capable of nitrosation in hydrophobic phases and [renitrosation] transnitrosation catalysts (denitrosilases).

Simultaneously, the compositions that allow achieving metabolism modulation of [nitric] nitrogen oxides according to different variants of the suggested method are claimed. Patent protection being claimed for the use for a new purpose of the known stabilized emulsions of inert fluorocompounds that are used as blood substitutes with gas transport function. In addition to accelerating NO oxidation those very compositions can be used for slowing down thereof. Thus the known blood substitutes based on fluorocompounds and possessing a gas transport function can be used for both accelerating (in small

relative volumes of the added hydrophobic compounds) and slowing down the process (in large volumes). The border between acceleration transitions into slowing down is determined by the equation for H acceleration value. Besides in clinical practice it is often necessary to remove the excess of NO and oxidation products thereof just from the aqueous phases (e.g. in septic shock when NO excess generated by macrophages results in a dangerous fall in blood pressure). Thus depending on the requirements in an individual--.

On page 12, cancel the second full and third paragraphs and insert therefore

--For modulating nitrogen oxide metabolism compositions comprising in addition to fluorocompounds modulating supplements including catalysts and/or [renitrosation] <u>transnitrosation</u> inhibitors (denitrosilases), nitrosation targets, reducers or combinations thereof are also claimed. The blood substitute "Perftoran" known before filing the previous application can be considered as a prototype for such compositions. The prototype drawback as regards the object of the given invention consists in an insufficient selectivity of modulating effect thereof i.e. impossible use thereof for varying (modulating) metabolism processes of [nitric] <u>nitrogen</u> oxides toward desired direction without changing NO oxidation rate.

The proposal has an inventive step since earlier the effect of micellar catalysis for changing the system of equilibration of [nitric] <u>nitrogen</u> oxides in NO oxidation was not known and N_2O_3 was

considered as an acting nitrosating intermediate [24]. Catalytic and inhibiting effect of the claimed [renitrosation] <u>transnitrosation</u> modulators (e.g. methionine- and histidine-comprising peptides, polyphosphates and metallic complexes thereof) and their contribution into the system modulation of [nitric] <u>nitrogen</u> oxides' equilibration was not known earlier. The composition in the form of emulsions the distribution curves by particle size of which--.

On page 13, cancel the first and second paragraphs and insert therefore

--have more than one maximum and/or prepared by mixing several compositions are also claimed. The advantage of such compositions consists in that each of the constituents can have an individual makeup and perform separate functions. For example, due to the fact that small micellae are phagocytized by some types of cells (e.g. macrophages) and large ones are not phagocytized by them, and alignment of component concentrations between individual micellae occurs rather slowly, metabolism of [nitric] <u>nitrogen</u> oxides in both macrophage producing large amounts of NO and in environment thereof can be controlled independently using such composition.

A method for effecting organism of a patient in need of correcting metabolism of [nitric] <u>nitrogen</u> oxides using the variants of the claimed method for modulating metabolism of [nitric] <u>nitrogen</u> oxides and/or compositions for realizing thereof is concurrently claimed.--

Cancel page 14 and insert therefore

--accelerated or slowed down as compared to the rate in absent perfluorocompounds. For this reason, higher [nitric] nitrogen oxides are predominantly formed in these novel phases and just they are the sources of NO-equivalents possessing various physiologic effects. The claimed methods for effecting organism of a patient use the capabilities of [nitric] nitrogen oxides' metabolism modulation method, i.e. of general or local modulating concentrations thereof in conformity with various cases. Since NO and nitrosothiols act as EDRF and participate in aggregation of platelets [5], the claimed methods can be used for preventing and treating ischemia, infarcts, strokes, blood coagulation pathology and hypertension. When preventing and treating atherosclerosis, one should take into consideration the fact that a lipid plaque on vascular wall also acts as a micellar NO oxidation catalyst and apoptosis modulator of surrounding cells [5]; therefore introduction of additional phases competing for a more efficient NO binding than that by tissue lipids is needed. Since theoretical upper limit for Q_{NO} in lipids is less than 70 [2] and in perfluorocompounds it is higher than 70, then the use thereof is particularly efficient. Along with the combinations of the known medicinal effects (NO donors, inhibitors of NO-synthases) on the metabolism of nitrogen oxides the methods based on the effects of change in temperature and/or humidity are claimed. This effect mechanism is also connected with NO oxidation activation and NO metabolism changes elicited thereby. Under hyperthermic conditions

organism protection against overheating consists in enhanced perspiration since cooling--.

On page 16 cancel the first paragraph and insert therefore --nitrite and nitrosothiols falls [5]. Decrease in concentration acts in opposite direction. Quantitative concentration relations for these processes are different. In hydrophobic phases of fluorine comprising emulsions change in oxygen concentration controls only NO oxidation rate. CO_2 is a modulator of NO oxidation, nitration and nitrosation since it destabilizes peroxynitrite while bicarbonate is a N_2O_3 hydrolysis catalyst. SF_6 is a biochemically inert gas well soluble in hydrophobic phases and modulating solubility therein of other gases in particular O_2 and CO_2. Solubility of nitrogen in hydrophobic phases is different from solubility of SF_6 and thus metabolism of [nitric] nitrogen oxides can be controlled by combination of partial pressures of these four gases. Air with NO admixture is used in clinical practice for ventilation and thus mixtures using all five gases (O_2, N_2, CO_2, SF_6 and NO) in combination with compositions of perfluorocompounds significantly expand capabilities of directed effect on metabolism of nitrogen oxides in various pathological conditions. Since lungs are one of the main organs of NO synthesis then a method using inhalation is claimed that can be used in treating bronchial asthma, hypoxia and pulmonary edema e.g. in mountain climbers.--.

On page 17 cancel the first two full paragraphs and insert therefore

--Figure 1. Relation between increase of NO oxidation rate by oxygen in a heterogeneous system (H) and the portion of the hydrophobic phase (x) and the [distribution] partition coefficient (Q) between phases. Both increase (the left arrow) and decrease in total oxidation rate at all phases are possible resulting from changes in x and/or Q.

Figure 2. Main metabolism reactions of [nitric] nitrogen oxides. NOS = NO-synthases, Hb = hemoglobin. Reaction 1 - NO biosynthesis by arginine oxidation, reactions 2-4 lead to irreversible removal of NO-equivalents from the cycle of [nitric] nitrogen oxides, reaction 5 - the process activation of which is possible with micellar catalysis. Steady state NO concentrations and oxidation products depend on the presence of [renitrosation] transnitrosation catalysts and concentration of targets for both nitrosation and binding free radicals. Reduction rates of nitrosothiols (RSNO) depend on R, presence of catalysts, reducers and on oxygen concentration.--.

On page 21, cancel the second full paragraph and insert therefore

--Thus in the both groups of experiments administering a new phase with a higher value of [distribution] partition coefficients Q into blood resulted in accelerated NO oxidation. In NAME group the own NO synthesis as affected by NO-synthases was inhibited and NO could be formed only in reducing nitrite and thionitrites (see Fig.2). Activation of NO oxidation with micellar catalysis in hydrophobic phases resulted in the growth of NO_2 concentrations and

therefore, nitrite concentration reduced and nitrate concentration raised. In the absence of NO-synthases' inhibitor--.

On page 22, cancel the third full paragraph and insert therefore

--The efficacy of [renitrosation] transnitrosation catalysts was determined by N^1-nitrozotriptophan (NOW) denitrosation kinetics in the composition of an acylic derivative or serum albumin at 32^{0}C and at different pH values by absorption decrease at ë = 340 nm or by differential spectroscopy relative to denitrosation reaction in a buffer solution, in relation to nitrite/nitrate ratio or by the ratio analysis of nitrosation products. Examples 7-17 illustrate catalytic and inhibitory activity of W^0/W^0_{buffer}--.

On page 26 cancel the third paragraph and insert therefore

--Example 36. A classic log Russian steam bath was used the bath being equipped with a hearth having a 100 liter capacity boiler located over the hearth. Stones fastened together by clay solution surround the hearth and the boiler. The bath is "black" stoked with birch firewood and ventilated for 1.5 hours after stocking. Average temperature on the bath shelf was 65^{0}C at 70% humidity; patients were exposed to 2 runs 12 minutes each in a stationary regimen with a 10-minute inter-run interval at 37^{0}C and 88% humidity. 150 ìl 0.1% aqueous solution of a peptide [renitrosation] transnitrosation catalysts comprising 80% Met-Glu-His-Phe-Pro-Gly-Pro and 20% Met(S=O)-Glu-His-Phe-Pro-Gly-Pro (the mixture of--.

IN THE CLAIMS:

57. A method for modulating metabolism of [nitric] nitrogen oxides by varying NO oxidation rate in a heterogeneous medium by changing makeup thereof **characterized in that** the number of phases in this medium and/or one or more volume ratios of phases and/or one or more NO or oxygen [distribution] partition coefficients between phases are modified in such a way that the values of the expression

$$H = \frac{1 + \sum_{i=1}^{i=n-1} \frac{k_i}{k_n} Q_{NO,i}^2 Q_{O_2,i} x_i - \sum_{i=1}^{i=n-1} x_i}{\left(1 + \sum_{i=1}^{i=n-1} Q_{NO,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)^2 \left(1 + \sum_{i=1}^{i=n-1} Q_{O_2,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)}$$

where H is acceleration of NO oxidation reaction with oxygen in heterogeneous n-phasic system as compared to the aqueous phase, k_i is reaction rate constant in i phase, Q_{Noi}, Q_{O2i} is a equilibrated partition coefficient of NO and O_2 in i phase, x_i is a portion of i phase in a total volume, change.

58. The method according to claim 57 **characterized in that** for accelerating NO oxidation with oxygen the changes are carried out in such a way that [the values of the expression

$$H = \frac{1 + \sum_{i=1}^{i=n-1} \frac{k_i}{k_n} Q_{NO,i}^2 Q_{O_2,i} x_i - \sum_{i=1}^{i=n-1} x_i}{\left(1 + \sum_{i=1}^{i=n-1} Q_{NO,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)^2 \left(1 + \sum_{i=1}^{i=n-1} Q_{O_2,i} x_i - \sum_{i=1}^{i=n-1} x_i\right)}$$

where H is acceleration of NO oxidation reaction with oxygen in heterogeneous n-phasic system as compared to the aqueous phase, k_i is reaction rate constant in i phase, Q_{Noi}, Q_{O2i} is a equilibrated partition coefficient of NO and O_2 in i phase, x_i is a portion of i phase in a total volume, change] H value would increase and for slowing down NO oxidation with oxygen the changes are carried out in such a way that the H value would decrease.

59. The method according to claim 57 **characterized in that** for changing NO [distribution] partition coefficients between the phases the medium quantitative makeup is changed without changing qualitative makeup and/or without forming novel phases.

62. The method according to claim 57 **characterized in that** the components being introduced comprise solution of a protein that solubilizes the fluorinated organic compound having the value of [distribution] partition coefficients Q_{NO} and/or Q_{O2} in a biphasic

system with water higher than the maximum value of Q_{NO} and/or Q_{O2} for arbitrary pair of phases of reaction mixture before introducing.

65. The method according to claim 57 **characterized in that** the components comprising one or more catalysts and/or [renitrosation] transnitrosation inhibitors are additionally introduced.

70. Compositions comprising a perfluororganic compound resistant in metabolic reactions and forming with water a heterogeneous mixture said compound being selected from the group including: 0.1 to 90% perfluorohydrocarbons, halo-derivatives of perfluorohydrocarbons, perfluoroalkylamines; 0.08% to 3.3% of one or more compounds belonging to one or more groups of the following list: catalysts or inhibitors of pernitrosifiation, reducers, scavengers of free radicals; and one or more compounds of the group: SF_6, perfluorohydrocarbons, halo-derivatives of perfluorohydrocarbons, tertiary perfluoroalkylamines, water up to 100% for modulating metabolism of [nitric] nitrogen oxides.

76. The compositions according to claim 71 **characterized in that** one or more substituted or unsubstituted mono- and/or di- and/or polyphosphates and/or complexes thereof with magnesium or zinc or copper or manganese are introduced as catalysts or [renitrosation] transnitrosation inhibitors.

77. The compositions according to claim 71 **characterized in that** one or more compounds of the group: thiourea, thioamides, methionine, arginine, peptides and/or acylic and/or amide dervatives thereof of general formula X-Pept-Y, where X=H or acyl, Y=OH or $-NH_2$ or NHR or

NR_1R_2, Pept=peptide comprising residues of methionine and/or aspartic acid and/or histidine and/or glutamic acid and/or arginine, are introduced as catalysts or [renitrosation]<u>transnitrosation</u> inhibitors.

86. A method for effecting organism of a patient in need of correcting metabolism of [nitric] <u>nitrogen</u> oxides **characterized in that** for modifying NO oxidation rates and subsequent reaction the number of phases is modified in the organism and environment thereof and/or one or more ratios between volumes of the phases and/or one or more [distribution] <u>partition</u> coefficients of NO or oxygen between the phases <u>in a manner that changes the acceleration of NO oxidation rate in heterogeneous n-phasic system as compared to the aqueous phase</u>.

89. The method according to claim 87 **characterized in that**

one or more compounds from the group:a perfluorohydrocarbon, a perfluorohydrocarbon halo-substituted derivative and a tertiary perfluoroalkylamine are introduced as the fluorine comprising water immiscible compounds;

copolymers of ethylene oxide and of propylene oxide and/or phospholipids are introduced as emulsifiers;

and/or glucose and/or fructose and/or saccharose are introduced as carbohydrates for maintaining osmotic pressure;

and/or ascorbic acid and/or salts thereof and/or retinol and/or acylic derivatives thereof are introduced as reducers;

and/or one or more substituted or unsubstituted mono- and/or di- and/or polyphosphates and/or complexes thereof with magnesium or zinc or copper or manganese are introduced as catalysts or [renitrosation] transnitrosation inhibitors;

and/or thiourea, thioamides, methionine, arginine, peptides and/or acylic and/or amide derivatives thereof of general formula X-Pept-Y, where X=H or acyl, Y=OH or $-NH_2$ or NHR or NR_1R_2, Pept=peptide comprising residues of methionine and/or aspargic acid and/or histidine and/or glutamic acid and/or arginine, and/or Pept comprises a fragment Met-Glu-His-Phe; and/or Pept = Met-Glu-His-Phe-Pro-Gly-Pro are introduced as catalystst or [renitrosation] transnitrosation inhibitors;

and/or tocopherol and/or acylic derivatives thereof are introduced as scavengers of free radicals;

and/or one or more thiols or dithiols or disulphides and/or one or more compounds from the group: dithiopropanol, dithiobutanol, lipoic acid, dihydrolipoic acid, cysteine, homocysteine, peptides comprising cysteine or cystine, acylic and/or esteric and/or amide derivatives of cysteine or cystine or peptides comprising these amine acids, or protein are introduced as the targets for nitrosation and/or precursors thereof;

and/or one or more compounds from the group: urea, glutamic, aspartic, carbamic, amidophosphoric, sulfamic acids and salts thereof, asparagine, glutamine, primary amine and salts thereof,

peptides comprising asparagine and/or glutamine are introduced as the targets for nitrosation with nitrogen release.

93. The method according to claim 86 **characterized in that** a patient is additionally administered catalysts or [renitrosation] transnitrosation inhibitors.

112. A use of steam bath or sauna for modulating metabolism of [nitric] nitrogen oxides by modifying NO oxidation rate and activation of biosynthesis thereof.